Corneal imaging of intrastromal femtosecond laser treatment for presbyopia (Intracor®)

Imagerie cornéenne du traitement intrastromal au laser femtoseconde pour la presbytie (Intracor®)

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Summary
Purpose. — To evaluate anatomic appearance and corneal cellular modifications after monocular Intracor® procedure with two different anterior segment imaging techniques within the first postoperative year.

Patients and methods. — Four patients underwent an Intracor® procedure in one eye performed at Clinique de la vision, and corneal imaging was performed in Quinze-Vingts National Ophthalmology Hospital. Slit-lamp photography, confocal microscopy with Heidelberg retinal tomography (HRT) and anterior segment spectral-domain optical coherence tomography (OCT) were performed 2 days, and 1, 6, and 12 months after the procedure.
Results. — Confocal microscopy showed strong cellular activation of keratocytes within the first postoperative month which diminished over time. The linear femtosecond incisions appeared as hyper-reflective regular lines and reflectivity decreased throughout the follow-up period. After 6 months, a fibrotic process with appearance of corneal scars was visible as small intrastromal hyper-reflective lines and thick hyper-reflective spicules around the incisions and remained stable over time. On spectral-domain OCT, the size and depth of the incisions decreased from the center to the periphery. Visibility of the corneal femtosecond incisions on OCT decreased from day 2 until they almost disappeared at 12 months. The incision angles seemed to curve progressively from the center to the periphery in their deep portion on the peripheral rings.

Conclusion. — Corneal cellular modifications found on HRT, anatomical features, and the sizes of the intrastromal rings, may provide valuable information on this new refractive technique.

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Introduction

Intrastromal corneal correction of presbyopia using a femtosecond laser treatment (Intracor®) is a new refractive technique developed by Luis Ruiz in 2007 [1]. The principle consists in changing the corneal curvature. To correct presbyopia, a multifocal hyperprolate cornea is created by generating a central corneal steepening with five femtosecond cuts. This modern refractive approach is a new, minimally invasive method with only femtosecond photodisruption and lacking any surface ablation. The corneal rings are purely intrastromal and do not affect the endothelium, Descemet’s membrane, Bowman’s layer, or the epithelium (Intracor. An excerpt from the presentations by Dr Luis Ruiz and Dr Mike Holzer and the roundtable discussion moderated by Dr Wing-Kwong Chan in the first Technolas Perfect Vision Alliance. 2009). A nomogram based on patient characteristics was used for the treatment parameters [2]. The purpose of this study was to evaluate tissular and cellular corneal modifications induced by these femtosecond laser circular cuts during a follow-up period of 12 months combining slit-lamp photography, Heidelberg retinal tomography (HRT) with confocal microscopy (CM), and anterior segment spectral-domain optical coherence tomography (SD-OCT).

Patients and methods

Patient demographics

Four eyes of four patients (two women and two men) were included in this case series (Table 1). Median age was 51 years (range: 47–71 years). Three left eyes and one right eye were operated. Median preoperative refraction in spherical equivalence for distance vision was +0.50 D (range: −0.25 D to +0.75 D) in the operated eye. Median preoperative refraction for addition in near vision was +2.25 D for the operated eye. All patients were examined at the Quinze-Vingts National Ophthalmology Hospital by the same examiner (MF). The study was performed at the Center of Clinical Investigations (CIC 503) of the Quinze-Vingts.
National Ophthalmology Hospital, with the approval of the Institutional Review Board of Saint-Antoine University Hospital (CPP-Ile de France 5, number 10793). All subjects were informed of the aims of the study, and their written informed consent was obtained.

The Intracor® procedure

The patients underwent a unilateral Intracor® procedure on the non-dominant eye. The Technolas® 520F femtosecond laser system (Technolas Perfect Vision GmbH, Munich, Germany) was used on these patients, delivering a laser pulse of 600–700 fs duration, at a 1053-nm wavelength, and a maximum of 6 μJ energy to achieve photodisruption within the cornea. Five concentric corneal rings were cut after marking the line of sight preoperatively. The different diameters of the five rings given by Technolas Perfect Vision are 1.8 mm, 2.25 mm, 2.7 mm, 3.15 mm, and 3.6 mm, respectively. The difference between the diameter of each adjacent ring is 0.45 mm. The femtosecond laser procedure used a pachymetry- and keratometry-reading-adjusted nomogram. The treatment time was approximately 20 s. Postoperative examinations were performed on day 2, month 1, month 6, and month 12.

Postoperative examinations

The ophthalmologic evaluation included uncorrected distance visual acuity (UDVA), corrected distance visual acuity (CDVA), uncorrected near visual acuity (UNVA), corrected near visual acuity (CNVA), slit-lamp biomicroscopy analysis, and a slit-lamp photograph of the cornea. All near reading tests were performed monocularly on the treated eye at a fixed distance of 40 cm from the treated eye under photopic conditions using near reading charts (catalog #2106, Precision Vision). All patients were then examined by the same operator (MF) with an SD-OCT (Spectralis OCT®, Heidelberg Engineering GmbH, Heidelberg, Germany) and HRT II/Rostock Cornea Module (HRT II/RCM; Heidelberg Engineering GmbH) confocal microscope, as follows: four eyes on day 2, four eyes at month 1, four eyes at month 6, and two eyes at month 12 after surgery.

OCT examination and image analysis

An SD-OCT fitted with an anterior segment module (Spectralis® OCT, Heidelberg Engineering GmbH, Heidelberg, Germany) was used. The OCT axial and lateral optical resolutions were 3.9 μm and 11 μm, respectively. All acquisitions were made using the high-resolution mode with an acquisition time of 19 ms per image. Since OCT is a noncontact technique, it was performed before the ophthalmologic examination and ocular surface tests to avoid potential artefacts. For each eye, the central cornea was analyzed. A horizontal scan was used for imaging of the central cornea to analyze the Intracor® incisions.

The images were analyzed with a 600–800% zoom factor provided by the Heidelberg Eye Explorer software (Heidelberg Engineering GmbH, Heidelberg, Germany) of the Spectralis® OCT. The five concentric Intracor® incisions were observed in the whole cornea. The depth of each incision and the distance between two incisions were measured on postoperative day 2 by two independent examiners who repeated every measurement five times and afterwards took the mean of each measurement using the cursors provided by the SD-OCT software.

Corneal confocal microscopy

The laser source used in the HRT II is a diode laser with a wavelength of 670 nm. The acquired two-dimensional image is defined by 384 × 384 pixels covering an area of 400 μm × 400 μm with lateral digital resolution of 1 μm/pixel and digital depth resolution of 2 μm/pixel. Before microscopy evaluation, one drop of a topical anesthetic, oxybuprocaine 0.4% (Oxybuprocaine®, Laboratoires Thea, Clermont-Ferrand, France), and one drop of a gel tear substitute, carbomer 0.2% (Lacnorm®, carbomer 980 NF; Bausch and Lomb, Rochester, NY, USA), were instilled in the lower conjunctival fornix. Each eye was examined for less than 5 min. No ocular surface changes related to the CM evaluation were noted with the Heidelberg Retina Tomograph/Rostock Cornea Module. The objective of the microscope was an immersion lens (Olympus, Hamburg, Germany), magnification × 60, covered by

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Table 1  Patient demographics, preoperative and 1-month postoperative data (Intracor® procedure).

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4 (First treatment)</th>
<th>Patient 4 (Second treatment)</th>
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</thead>
<tbody>
<tr>
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<td>71</td>
<td>47</td>
<td>53</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>Sex</td>
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<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
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<tr>
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<td>R</td>
<td>L</td>
<td>L</td>
<td>L</td>
<td>L</td>
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<tr>
<td>Preoperative SE (D)</td>
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<td>0.5</td>
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<td>−0.25</td>
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<tr>
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<td>20/32</td>
<td>20/20</td>
<td>20/32</td>
<td>20/32</td>
</tr>
<tr>
<td>Postoperative UDVA (Snellen)</td>
<td>20/32</td>
<td>20/32</td>
<td>20/25</td>
<td>20/32</td>
<td>20/32</td>
</tr>
<tr>
<td>Postoperative UNVA (Snellen)</td>
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<td>20/50</td>
<td>20/50</td>
<td>20/25</td>
</tr>
<tr>
<td>Postoperative Myopic shift</td>
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<td>−0.25</td>
<td>−0.25</td>
<td>−0.25</td>
<td>−0.50</td>
</tr>
</tbody>
</table>

F: female; M: male; R: right; L: left; SE: spherical equivalent refraction; UNVA: uncorrected near visual acuity; CDVA: corrected distance visual acuity; D: dioptres.
a polymethylmethacrylate cap. The focal plane of analysis could be displaced manually over the entire cornea.

The examination was performed in the sagittal axis (anteroposterior axis) so that, as the operator proceeded, the corneal epithelium, subepithelial neural plexus, anterior stroma, posterior stroma, and endothelium were successively examined. For all eyes studied, several confocal microscopic images were taken of all corneal layers where incisions induced by femtosecond laser were observed. Images were analyzed retrospectively by two examiners (LT, AL) in a masked manner to compare corneal patterns. In vivo confocal corneal microscopy images were then correlated to clinical refractive results. The thickness of incisions and the distance between each incision were measured for each patient on five different images at each examination by GIMP software (version 2.6.11), and the means of these values at each examination were calculated for all incisions, after which statistical tests were performed. In vivo confocal microscopy images were evaluated for reflectivity with the gray value of corneal incisions and their surrounding area by ImageJ software (version 1.43u, National Institutes of Health, Bethesda, MD, USA). A line was drawn on the incision zone on IVM images to yield the reflectivity profile of the incision. The software then gave a median value of reflectivity of the zone analyzed related to its surface. For each examination of each patient, five different images were analyzed separately and a median value was calculated from these five images.

Statistical analysis

Results for the descriptive statistics are presented as median and range. Variance was analyzed using the Kruskal–Wallis test, and the data were then compared with the Mann–Whitney statistical test. The differences were judged significant when \( p < 0.05 \).

Results

Refraction and visual acuities

In the four patients, from the first month after surgery until the last examination, clinical refractive results were stable. For the operated eye, two patients had an UNVA of 20/25 (patients 1 and 2) and two had an UNVA of 20/50 (patients 3 and 4). All patients had a myopic refractive shift. Spherical shift for CDVA at the 1, 6 and 12 months was stable and its median was \(-0.50 \)D (range: \(-0.25 \) to \(-0.75 \)). Preoperative spherical equivalent refraction, preoperative UDVA, postoperative UDVA, postoperative UNVA, postoperative myopic shift for CDVA are presented in Table 1. Patient 4 had a second similar treatment in the same eye (procedure called Re-COR® centered on the first treatment) 4 months after the first Intracor® surgery because of an unsatisfactory increase of UNVA. After the Re-COR® procedure, there was a loss in UDVA (20/32 to 20/50), while the patient had an increased UNVA (20/50 to 20/25) (Table 1). The two different Intracor® treatments for this patient were studied separately over time.

For all patients, at slit-lamp examination, the outline of the five concentric corneal rings was strongly delimited on postoperative day 2 and gradually blurred after that until 12 months, but was still visible at the last examination. The outline of the central rings became white (Fig. 1 left and middle). The second treatment for patient 4, centered on the first Intracor®, is shown in Fig. 1 right.

Corneal imaging

Using SD-OCT, the five concentric rings could be analyzed with measurements of their depth, size, and distance to the endothelium and epithelium on day 2 (Fig. 2). The size and depth of incisions decreased from the center to the periphery. The median distance between two rings was 0.22 mm (range: 0.21–0.24 mm). There was no statistical difference between the peripheral and central rings for this distance (data not shown). The incision angles were not totally perpendicular to the corneal surface but seemed to curve progressively from the center to the periphery in their deep portion on the peripheral rings (Fig. 2). In all patients, the incision outline was well defined right after surgery (day 2) and still visible at 1 month, but was then slightly blurred on OCT after 6 months (Fig. 3) until 12 months.

The CM images could be obtained in each layer from the central corneas of all patients examined. The femtosecond laser-induced incisions were observed and appeared as hyper-reflective regular circular lines (Fig. 4 top left).
Figure 2. Spectral-domain OCT (SD-OCT) image of cornea 2 days after intrastromal femtosecond laser treatment for presbyopia (Intracor®). The size of the femtosecond laser-induced incision, the epithelium–incision and incision–endothelium distances were assessed for the five concentric rings. Angles of incisions curved in a centrifugal way in the posterior portion of the most peripheral rings (arrow).

Figure 3. Spectral-domain OCT (SD-OCT) images of cornea 2 days (top), 1 month (middle), and 6 months (bottom) after Intracor® treatment. Hyper-reflectivity of incisions decreased progressively until 6 months.

median of thickness was 12.5 \mu m (range: 9.5–15.1 \mu m). Median distance between two incisions measured by CM was also 0.22 mm (range: 0.19–0.27 mm). There was no statistical difference between each time of examination for these values. The outline of these rings progressively blurred from the first month until the last examination (Fig. 4 top right, bottom left and bottom right), while incision reflectivity (measured on Fig. 5) gradually decreased at the same time (data not shown). In all patients, on day 2 a strong activation of keratocytes was shown with high reflectivity. These keratocytes were identified with visible cytoplasmic processes and bright nuclei (Fig. 6 top left) inside an edematous area visible as stromal extracellular fluid cystic spaces. Activation seemed to be most significant on the day-2 examination. At the 1-month examination, keratocytic activation diminished, and at the 6- and 12-month examinations, inactive, normal keratocytes were observed. Moreover, at the same time intervals, fibrillar processes were observed. In two patients, the incisions appeared as a rail (Fig. 6 top middle). In all patients, after 6 months, corneal scars appeared in the stroma as needle-like patterns, and were still visible after 12 months (Fig. 6 top right). Thick hyper-reflective spicules around the incision (Fig. 6 bottom left) might represent another cicatrizing sign after femtosecond laser treatment. Crossing of two consecutive Intracor® treatments could be imaged with CM (Fig. 6 bottom middle), and the reflectivity of the two different aged incisions could be easily distinguished. In one patient, we detected small, distinct, regularly aligned dots, which could be femtosecond laser impacts (Fig. 6 bottom right). We did not find any correlation between CM features and refractive results in these four patients.

Discussion

Intrastromal femtosecond laser treatment for presbyopia (Intracor®) is a new surgical technique used to induce a multifocal cornea via generating a central corneal steepening. However, some reports have been published in the literature [1–9], and little information was provided by the company to explain the refractive procedure, except for the diameters of the rings. Corneal imaging used in this study provided a precise anatomical description of the concentric rings at tissular scale with SD-OCT and allowed us to evaluate the cellular reaction and corneal modifications around the incisions with confocal microscopy. Both SD-OCT and CM examinations confirmed the official information given by the firm for the distance between each ring, as we found a distance of 0.22 mm between each ring with both techniques. The SD-OCT images helped us to understand the refractive mechanism based on the decreasing size and depth of the concentric rings from the center to the periphery.
Figure 4. Confocal microscopy (CM) images (400 × 400 μm) of the cornea of patients after intrastromal femtosecond laser treatment for presbyopia (Intracor®). The femtosecond laser-induced incision appeared as a hyper-reflective regular circular line (top left) 2 days postoperatively. At 1 month (top right), 6 months (bottom left) and 12 months (bottom right), the outline of incision gradually blurred.

Figure 5. Evaluation of the reflectivity of intrastromal femtosecond laser incisions on confocal microscopy (CM) images. A white line delineated a zone centered on the incision (arrow) on the CM image (left) on which the ImageJ software gave a reflectivity plot (right) in gray value.

and to the curving angle of these rings in a centrifugal way. Angulation of the incision has already been described (Guedj T, Danan A, Lebuisson DA. Imagerie Intracor. Clinique de la Vision - Des Chirurgies Réfractives 2010, Special Edition No 4; April 2010: 26–30; Albou-Ganem C, Amar R, Pages C, et al. La correction intrastromale de la presbytie par la technique IntraCor. Mode d’action et résultats à 1 an. Clinique de la Vision - Chirurgies Réfractives 2011, Special Edition No 5; April 2011: 58–62). Ganem et al. explained the deviation of incisions by the corneal returning to its natural curvature after the end of applanation. Moreover, the posterior part of the incision might deviate due to the centrifugal slip of the posterior cornea. Ganem et al. suggested that the central ring is less affected by the curvature of the incision.
because of less applanation. Peripheral rings show greater deviation because their localization in the posterior stroma exposes them to more shear force. The confocal microscopic examination analyzed the femtosecond laser-induced rings at the cellular scale, and these incisions could be described as hyper-reflective regular lines. The rail seen in Fig. 6 top middle, with two hyper-reflective lines separated by a hyporeflective strip, could be explained by a double row of impacts or a cleavage of the incision in two parts by the femtosecond laser. The corneal reaction around the rings is consistent with findings from other studies on the activation of keratocytes induced by femtosecond laser treatment. Indeed, Sonigo et al. showed hyper-reflective nuclei and processes evocative of keratocyte activation under the LASIK interface with a femtosecond laser [10,11]. Keratocyte activation and interface backscatter were positively correlated with the raster energy of the femtosecond laser used during surgery [12]. Keratocyte activation with hyper-reflective nuclei and processes was described in eyes of rabbits after femtosecond laser keratotomy [13], femtosecond laser corneal flap [14], and femtosecond laser-assisted multi-layer intrastromal ablation [15]. In a study where patients benefited from femtosecond laser arcuate keratotomy for the correction of high astigmatism after keratoplasty [16], in vivo confocal microscopy found keratocyte activation along the incision edges with edematous reaction, followed by stromal fibrotic scarring, as in the present study, and anterior segment OCT described the incision as a hyper-reflective linear pattern extending from the subepithelial to the pre-endothelial layers. Another similar confocal microscopic image caused by femtosecond laser treatment is the presence of small, distinct, regularly aligned dots also interpreted in other publications as femtosecond laser impacts [13] or as the tissular response of these impacts [11]. We also noted these dots in one patient. Global reduction of the reflectivity of the incisions with time on SD-OCT and on CM corroborated the clinical examination on slit-lamp photographs, showing that the outline of the peripheral rings had receded, but the white color of the central rings observed on slit-lamp photography after 6 months can be explained by fibrotic scars shown on CM around the incisions. The needle-like hyper-reflective patterns observed after 6 months in all patients looked like infectious crystalline keratitis examined with confocal microscopy, which were nonspecific to an infectious agent [17]. There was no sign of infection in our Intracor® series. These small, thin, needle-like hyper-reflective lines also resembled corneal scars described after excimer laser photorefractive keratectomy [18], and confirmed the corneal fibrotic process at this time. This fibrotic phenomenon can explain the stability of refractive effects on the cornea we noticed 1 year after treatment. Recent studies also confirmed the stability of gain of UNVA and corneal steepening up to 12 and 18 months postoperatively [8,9].
The second Intracor® treatment centered on the first one (called Re-COR®) has already been described. It was indicated for unsatisfied patients after one Intracor® procedure to increase the multifocal effect on the cornea. Pages et al. observed better near vision for all patients who benefited from the Re-COR® protocol with a larger myopic shift, like patient 4 in our series (Pages C, Guedj T. Le retraitement après insuffisance de correction par une procédure IntraCor réelle et bien centrée : le protocole Re-Cor. Clinique de la Vision-Chirurgies Réfractives 2011, Special Edition N° 5; April 2011: 63–66). This myopic shift (of 0.5 D in our case series) is the most understandable mechanism of the Intracor® procedure for the improvement of uncorrected near visual acuity, but it is probably accompanied by poorer distance vision, as in patient 4. Myopic shift of 0.6 D was also described in other articles with loss of CDVA in some cases [8, 9]. This is why patients selected for this surgery have to be slightly hyperopic to improve their far and near vision after this myopic shift.

Intrastromal femtosecond laser treatment for presbyopia (Intracor®) produced, thanks to five concentric rings, a refractive effect with multifocality [2]. In the current work, we assessed:

- the anatomic aspects of these rings to understand the mechanism of this new treatment;
- the cellular corneal modifications caused by the femtosecond laser in a 12-month follow-up period.

The two major findings in CM were activation of kerocytes within the first postoperative month diminishing over the time and appearance of corneal scars, stable over the time.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

**References**


